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Diagnostic white blood cells can be a predictor for mutation existence in myelodisplastic syndromes: the role of next generation sequencing

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ABSTRACT

Aims: Myelodisplastic syndromes (MDS) are hematological disorders originating from clonal damage and characterized by inefficient bone marrow activity. The existence of mutations has been shown to play a significant role in risk, treatment response, turnover to acute myeloid leukemia, prognosis, and overall survival. Next-generation sequencing (NGS) is a technique that detects gene mutations and has been the subject of studies for detecting MDS-related mutations. We aimed to investigate the importance of the NGS method and identify mutation-related blood parameters in MDS patients.

Methods: We conducted a study on 33 MDS patients during the period of 2021–2023. Patients were analyzed for the existence of mutations with the NGS technique, and the hematological parameters, transfusion need, IPSS, and IPSS-R scores of these patients were recorded.

Results: Fifteen out of 33 patients (45.5%) were tested for mutations. White blood cell (WBC) levels at the diagnosis of patients with type 1 number somatic mutations were higher than in other groups, 7056.25, 2416.67/µL, and 3036.67/µL, respectively (p=0.048). The ratio of neutrophil and lymphocyte values recorded and NGS positive were higher than those tested negative (1.56 and 3.51, respectively). Patients with the type 1 mutation had higher WBC levels than patients with the type 2 and type 3 mutations, 7056.25/µL, 2416.67/µL, and 3036.67/µL, respectively.

Conclusion: Genetic mutations are common in MDS patients, and NGS is a useful tool to detect them. Understanding the role of mutations in the matter of risk classification, treatment response, and prognosis is quite important, and larger-scale studies are needed.

Keywords: MDS, myelodisplastic syndromes, NGS, next generation sequencing

INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal bone marrow stem cell disorders associated with inefficient haematopoiesis resulting in blood cytopenias and are characterized by progression to acute myeloid leukemia in one third of patients.[1] Differential diagnosis of MDS can be challenging due to its heterogeneous nature and subjective assessment of dysplasia. The correct diagnosis and classification of MDS depends on an accurate assessment of both clinical features and laboratory or pathological findings (e.g., blast count, peripheral blood count, cytogenetics). Chromosomal abnormalities have been reported in 40% to 70% of patients with MDS and in the majority of patients with treatment-related MDS.[2]

Diagnosis of MDS can be challenging in patients with uninformative cytogenetics, a normal karyotype, or who lack robust morphological markers such as ring sideroblasts or myeloblast abundance. Important studies are underway to identify new diagnostic tools that can make the diagnosis of MDS more accurate.[3] Next-generation sequencing (NGS) is also being studied. NGS is a cytogenetic method used in diagnosis and treatment. By using targeted gene panels with NGS, it has become possible to diagnose many mutations simultaneously and to detect rare variants and changes at the chromosomal level much more sensitively than the traditional polymerase chain reaction (PCR) method.[4]

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In borderline MDS and acute myeloid leukemia, where the blast rate is close to 20%, the use of NGS, in addition to other tests that detect genetic mutations, is beneficial for diagnosis. Studies have shown that NGS may be useful in the prognostic evaluation of MDS cases with persistent or refractory cytopenia and normal cytogenetics.[5] Studies have shown that TP53, SRSF2, and TET2 mutations detected by NGS are poor prognostic factors, especially SRSF2 mutations, which may accelerate the transformation of myeloproliferative neoplasms to acute myeloid leukemia.[6]

In our study, we investigated the factors influencing overall survival in MDS patients, the factors influencing the disease process when evaluating response in terms of transfusion, blasts, and fibrosis, the role of the presence of abnormalities and number somatic mutations as a result of NGS in treatment response and overall survival, and the parameters at the time of diagnosis (white blood cell (WBC), neutrophils, lymphocytes, neutrophil/lymphocyte ratio, hemoglobulin (Hb)). The aim is to determine the relationship between NGS results (e.g., platelets, blasts, etc.) and the prognostic significance of these parameters.

METHODS

Patient Selection

Our study included 33 patients (16 male and 17 female) diagnosed with MDS who were admitted to Konya City Hospital between 2021 and 2023. The ethics committee required for the study was obtained from the Hamidiye Scientific Researches Ethics Committee (Date:18.04.2024, Decision No: 5/21). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients who were older than 18 years of age, diagnosed with MDS, and had NGS and number somatic mutations data were included in the retrospectively designed study.

Statistical Analysis

Statistical analyses were performed using the "IBM SPSS Statistics for Windows Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)" program. Descriptive statistics are presented as frequency and % for categorical variables, mean±standard deviation, and median (IQR) for continuous variables. The data of the study were evaluated with normality assumptions using the Kolmogorov-Smirnov normality test. An independent sample t test, or Mann-Whitney U test, was used for comparisons between two independent groups. The Kruskal-Wallis H test was used for comparisons of three or more independent groups, and the Mann-Whitney U test with Bonferroni correction was applied for subgroup comparisons. Comparisons of categorical variables were made using the chi-square test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Thirty-three patients were included in the study, and the mean age was 70.21 years. Seventeen of them were male. In fifteen patients NGS test resulted positive. Number somatic mutations analysis showed that nineteen of the patients had 0, eight of them had 1, and the rest of the patients were distributed equally between 2 and 3 mutation types. Fifteen patients were treated with hypomethylating agents (HMA), and fourteen patients were treated with erythropoiesis-stimulating agents (EPO). Most of the patients (n=25) did not receive second-line treatment. Eight of the patients showed a response in blast levels. In fifteen of the patients, it is noted that the blood transfusion need was lowered. The mean WBC levels of patients at diagnosis were 5313.64/µL. The ratio of neutrophil and lymphocyte levels at diagnosis in the patients was noted at 2.62 (Table 1) .

Table 1. Characteristic features of the patients included in the study

Characteristics	
Age (years) (mean±SD)	70.21±11.43
Sex	
Male	16 (48.5)
Female	17 (51.5)
Treatment (n (%))	
HMA	15 (45.5)
Epo	14 (42.4)
Immune modulator	3 (9.1)
None	1 (3)
Second line treatment (n (%))	
HMA	2 (6.1)
Immune modulator	4 (12.1)
HMA+Venetoclax	2 (6.1)
None	25 (75.8)
Response in blast levels (n (%))	
Positive response	8 (24.2)
Negative response	5 (15.2)
No response	20 (60.6)
Response according to blood transfusion need (n(%))	
Decrease in transfusion need	15 (45.5)
No change in transfusion need	16 (48.5)
Unrelated to transfusion need	2 (6.1)
Anomalia in NGS (n(%))	
Present	15 (45.5)
None	18 (54.5)
Number somatic mutations (n(%))	
0	19 (57.6)
1	8 (24.2)
2	3 (9.1)
3	3 (9.1)
Follow up (months)	
NGS Anomaly	**Mean±SD**
Positive	13.20±4.70
Negative	18.11±12.92
Number somatic mutations (n) (mean±SD)	
0	18.84±12.47
1	13.12±3.27
2	9.66±4.72
3	10.66±0.57
SF3B1	5
ASXL1	3
TP53	3
SRSF2	2
DNMT3A	2
TET2	2
RUNX1	2
IDH2	1
NPM1	1
SETBP1	1
U2AF1	1

Abbreviations: HMA: Hypomethylating agent, Epo: Erythropoiesis stimulating agents, WBC: White blood cells, RBC: Red blood cells, HGB:Hemoglobin, SD: Standard deviation, NGS: Next generation sequencing

The WBC levels of patients according to positive and negative NGS results were 4972.67/μL and 5597.78/μL, respectively. Neutrophil and lymphocyte levels of NGS-positive patients were 2467.11/μL and 1487.33/μL, respectively. These values were 3545.55/μL and 1343.89/μL for NGS-negative patients, respectively. The ratio of neutrophil and lymphocyte values for the NGS positive and negative groups was 1.56 and 3.51, respectively. There was no significant difference between patients due to the presence of an NGS abnormality. However, the neutrophil/lymphocyte ratio at diagnosis was found to be lower in patients with NGS abnormalities (p=0.062). Eleven types of mutations were detected in NGS analyses. In five patients, the SF3B1 mutation was detected. ASXL1 and TP53 mutations were the most common mutations in each of the 3 patients (**Table 2**).

Table 2. Comparison of values recorded at diagnosis of MDS patients according to NGS anomaly group

Variables	NGS+(mean+SD)	NGS-(mean+SD)	p
WBC (10^3/μL)	4972.67±3295.58	5597.78±2507.30	0.541
RBC (x10^6/μL)	374.30±1090.99	374.30±1090.99	0.198
HGB (g/dl)	8.56±1.92	9.12±2.06	0.423
MCV (Fl)	101.39±12.99	97.90±16.45	0.511
Neutrophil (10^3/μL)	2467.11±2433.11	3545.55±2354.80	0.206
Lymphocyte (10^3/μL)	1487.33±788.76	1343.89±553.25	0.545
Neutrophil/Lymphocyte	1.56±1.03	3.51±3.76	0.062
Platelet (10^3/μL)	188.13±124.39	204.28±104.13	0.688

Abbreviations: MDS: Myelodisplastic syndromes, WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, MCV: Mean corpuscular volume, SD: Standard deviation

As seen in **Table 3**, there is a statistically significant difference between the WBC levels of MDS patients at diagnosis and the number somatic mutation group averages (p=0.048). Accordingly, it is possible to say that the mean of the patients with type 1 mutation for the WBC levels of MDS patients at diagnosis was greater than the mean of the 2 and 3 types of mutations, 7056.25/μL, 2416.67/μL, and 3036.67/μL, respectively. No statistically significant difference was found between the somatic mutation group averages of other variables in MDS patients (p>0.005).

There was a statistically significant difference in the platelet values recorded at diagnosis according to the transfusion needs of MDS patients (p=0.014).

Accordingly, it is possible to say that the mean of the diagnostic platelet variable in the group with no change in transfusion need is higher than the mean of the group with a change in transfusion need, 237.70/μL and 121.13/μL, respectively (p<0.05). No statistically significant relationship was found between the group averages of other variables for MDS patients (p>0.05).

Response was evaluated by treatment type for transfusion need, blast levels, and fibrosis status (**Table 4**). While a decrease in blast rate was observed in those treated with HMA (p=0.002), no statistically significant difference was detected between all 3 treatment types in terms of response to regression in fibrosis score and reduction in transfusion need. It was observed that there were more people who responded at the blast level with HMA treatment, those who did not show a change at the blast level with EPO treatment, and those who did not respond at the blast level with immune modulator treatment. No statistically significant relationship was found between the type of treatment and changes in transfusion and fibrosis levels in MDS patients (p>0.05).

Table 4. Comparison of blood levels of parameters of MDS Patients at diagnosis according to groups created based on blast level responses

Parameters	Response in blast levels	Mean±SD	p*
WBC	No response	6626.00±2729.55	0.097
	Response	3683.75±2233.58	
	No change	5637.50±2950.78	
RBC	No response	2.93±0.61	0.987
	Response	2.98±1.14	
	No change	336.97±1038.35	
HGB	No response	9.24±1.84	0.851
	Response	9.01±2.13	
	No change	8.72±2.04	
MCV	No response	96.58±14.63	0.877
	Response	99.12±15.08	
	No change	100.36±15.49	
Neutrophil	No response	2659.35±2004.48	0.201
	Response	1913.75±1712.54	
	No change	3611.00±3642.53	
Lymphocyte	No response	1096.00±329.74	0.618
	Response	1353.75±567.55	
	No change	1509.50±749.60	
Neutrophil / Lymphocyte	No response	2.44±2.10	0.376
	Response	1.46±1.11	
	No change	3.14±3.58	
Platelet	No response	155.20±85.45	**0.014**
	Response	121.13±62.36	
	No change	237.70±116.69	

Abbreviations: MDS: Myelodisplastic syndromes, WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, MCV: Mean corpuscular volume, SD: Standard deviation

A statistically significant difference was found between the blast levels of MDS patients and the averages of the IPSS and IPSS R scores (p<0.05) (**Table 5**).

When the data were examined according to the international prognostic scoring system (IPSS) and revised international prognostic scoring system (IPSS R) variables, it was determined that there were statistical differences in the blast level, and this difference was highest in those with responses at blast levels of 0.75 and 4.87, respectively. No statistically significant

Table 3. Comparison of values of MDS patients according to number somatic mutations group

Variables (mean+SD)	0	1	2	3	p
WBC (10^3/μL)	5396.84±2444.91	7056.25±3351.11	2416.67±1363.46	3036.67±1876.17	**0.048**
RBC (x10^6/μL)	354.75±1063.68	2.81±0.42	1.97±0.73	3.13±0.83	0.276
HGB (g/dL)	9.08±2.11	8.97±1.25	6.60±1.66	9.46±2.43	0.303
MCV (Fl)	98.27±16.31	99.47±12.20	110.30±19.45	96.43±6.02	0.700
Neutrophil (10^3/μL)	3289.47±2310.18	3723.34±3017.92	1160.00±662.04	1686.66±1574.55	0.226
Lymphocyte (10^3/μL)	1397.89±623.67	1682.50±796.75	1026.67±786.21	1133.33±258.13	0.484
Neu/Lym	3.29±3.74	1.99±1.07	1.23±0.40	1.53±1.60	0.628
Platelet (10^3/μL)	229.05±121.39	146.13±93.23	200.67±90.25	125.33±50.29	0.206

Abbreviations: MDS: Myelodisplastic syndromes, WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, MCV: Mean corpuscular volume, SD: Standard deviation

Table 5. Relationship between treatment type and response to treatment and blast response in MDS patients

	Response in transfusion levels					
	Unrelated to transfusion	Decrease in transfusion need	No change	χ^2	SD	p*
No treatment	0	0	1	1,752	6	0,941
HMA	1	7	7			
EPO	1	7	6			
Immune modulator	0	1	2			
	Response in blast levels					
	No response	Response	No change	χ^2	SD	p*
No treatment	0	0	1	20,499	6	**0,002**
HMA	2	8	15			
EPO	1	0	14			
Immune modulator	2	0	3			
	Response in fibrosis levels					
	No response	Response	No change	χ^2	SD	p*
No treatment	1	0		2,142	3	0.544
HMA	13	2				
EPO	10	4				
Immune modulator	3	0				

Abbreviations: MDS: Myelodisplastic syndromes, HMA: Hypomethilating agent, Epo: Erythropoiesis-stimulating agents, χ^2: Chi square, SD: Standard deviation

difference was found between the blast levels of MDS patients and the average follow-up period (p>0.05).

A statistically significant relationship was found between IPSS R risk scores and blast levels in MDS patients (p=0.017). IPSS R risk scores are categorized as very low, low, intermediate, high, or very high. Accordingly, those with no response in blast values were at the expected level; risk scores in those who responded were very low and low; and in those with no change, intermediate, high, and very high values were found to be lower. No statistically significant relationship was found between IPSS R risk scores of MDS patients and transfusion requirement and fibrosis levels (p>0.05) (**Table 6**).

No statistically significant difference was found between the average follow-up period of the NGS Anomaly variable and that of MDS patients (p=0.142) (**Table 7**).

Table 6. Comparison of responses in blast levels of MDS patients with IPSS, IPSS R and follow-up period

Parameters	Response in blast levels	Mean±SD	p*
IPSS	No response	0.40±0.41	**0.027**
	Response	0.75±0.46	
	No change	0.27±0.49	
IPSS R	No response	3.00±0.93	**0.002**
	Response	4.87±1.90	
	No change	2.27±1.30	
Follow-up (month)	No response	14.00±5.61	0.562
	Response	13.25±4.39	
	No change	17.40±12.49	

Abbreviations: MDS: Myelodisplastic syndromes IPSS: International prognostic scoring system, IPSS-R: Revised international prognostic scoring system, SD: Standard deviation

Although there was no significant difference, the mean follow-up time for those with NGS anomalies was higher than for those without, at 18.11 and 13.20 months, respectively. No

Table 7. Relationship between IPSS R risk scores of MDS patients and transfusion need, blast and fibrosis levels

		Response in transfusion need					
		Unrelated to transfusion	Decrease in transfusion need	No change	χ^2	SD	p
IPSS R risk	Very low	0	3	3	12,578	8	0,127
	Low	1	6	10			
	Intermediate	0	2	3			
	High	1	1	0			
	Very high	0	3	0			
		Response in blast levels					
		Negative response	Positive response	No change	χ^2	SD	p
IPSS R risk	Very low	0	0	6	18,551	8	**0,017**
	Low	3	2	12			
	Intermediate	2	2	1			
	High	0	2	0			
	Very high	0	2	1			
		Response in fibrosis levels					
		Negative response	Positive response		χ^2	SD	p
IPSS R risk	Very low	6	0		3,699	4	0.448
	Low	13	4				
	Intermediate	4	1				
	High	1	1				
	Very high	3	0				

Abbreviations: MDS: Myelodisplastic syndromes, IPSS R: Revised international prognostic scoring system , χ^2: Chi square, SD: Standar deviation

statistically significant difference was found between the number of somatic mutations in MDS patients and the average follow-up time (p<0.064). Although there is no statistically significant difference here, it is possible to say that those with a somatic mutation number of 2 have the lowest follow-up period, and those with a somatic mutation number of 0 have the highest (**Table 8**).

Table 8. Comparison of NGS anomaly and number somatic mutations data and follow-up periods of MDS patients

Follow up (months)		
NGS anomaly	**Mean±SD**	**p***
Positive	13.20±4.70	0.142
Negative	18.11±12.92	
Number somatic mutations		
0	18.84±12.47	0.064
1	13.12±3.27	
2	9.66±4.72	
3	10.66±0.57	

Abbreviations: NGS: Next generation sequencing, SD: Standar deviation, MDS: Myelodisplastic syndromes

DISCUSSION

Understanding genetic manifestations of hematological cancers has been the subject of studies lately, and NGS studies have been the subject of studies.[6] We conducted a study to determine the role of NGS positivity in the prognosis of MDS patients. Thirty-three MDS patients were included in the study, and 15 of them were NGS-positive. Patients were examined according to WBC levels at diagnosis. WBC levels at the diagnosis of patients with type 1 somatic mutations were higher than in other groups (7056.25, p=0.048). The ratio of neutrophil and lymphocyte values for NGS positives was higher than those tested negative (1.56 and 3.51, respectively). Patients with the type 1 mutation had higher WBC levels than patients with the type 2 and type 3 mutations (7056.25/µL, 2416.67/µL, and 3036.67/µL, respectively). Patients who did not show a change in transfusion need during the illness had higher platelet levels at diagnosis than patients with transfusion need 237.70/µL and 121.13/µL, respectively (p<0.05). Patients with NGS positivity had a longer follow-up period than the negative group (18.11 and 13.20 months, respectively).

In a study conducted by Li et al.[6] in a univariate analysis, BCORL1 was shown to be an important parameter for the OS in patients with MDS diagnosis. Yang et al.[7] used optical genome mapping and conducted comprehensive cytogenetic scoring system analysis on 101 MDS patients. They discovered that the TP53 mutation and bone marrow blasts were important parameters for the prediction of survival. In our study, we detected the TP53 mutation in three patients. In a study examining MDS patients on eltrombopag and azacitidine therapy, NGS results revealed that patients with TP53, NRAS, ASXL1, RUNX1, or TET2 mutations were more prone to progress and less susceptible to eltrombopag therapy.[8] In our study, ASXL1, RUNX1, and TET2 mutations were detected in 3, 2, and 2 patients, respectively.

NGS positivity is common in MDS patients. In a study conducted on 95 MDS patients, 91.4% of the patients tested positive for at least one mutation.[9] MDS is a clonal stem cell disorder that develops due to a complex genetic process caused by DNA damage with subtypes. Understanding the route of the disease is quite important since the MDS can turn into acute myeloid leukemia. NGS has been shown to be a useful tool for diagnosis, classification, and surveillance of treatment types.[10]

The role of mutations in treatment response in MDS patients has been the subject of studies. In an analysis made on bone marrow specimens of MDS patients, the presence of T cell receptor repertoire differences was correlated with the response to HMA treatments.[11] Takahashi et al.[12] studied the genes of 114 untreated MDS patients and concluded that the existence of four or more driver mutations was linked to poor response to HMA treatments.

It is clear to say that mutations are common among MDS patients, and the clinical significance of them is still the subject of studies. Since the diagnosis and prognosis of MDS and treatment responses to therapies require attention for better clinical outcomes, new technologies remain important for the process.[13] In a study that compares NGS with cytogenetics, NGS showed better diagnostic performance, and it is noted that karyotyping can be reduced by 30% by using NGS as a first-line approach.[14] We identified mutations in MDS patients using NGS and found a correlation with WBC levels. Therefore, patients with lower WBC levels should be tested for mutations.

CONCLUSION

We studied thirty-three MDS patients with NGS techniques for detecting mutations. Fifteen (45.5%) of the patients were positive for at least one mutation. Patients with NGS positivity had lower WBC levels than negative ones. Since MDS has a nature of clonal damage and mutations, understanding the route of the disease and planning treatments according to mutation types is quite important. Studies with larger populations should be conducted, and the use of this technique must be implemented in diagnosis and treatment processes.

ETHICAL DECLARATIONS

Ethics Committee Approval

The ethics committee required for the study was obtained from the Hamidiye Scientific Researches Ethics Committee (Date:18.04.2024, Decision No: 5/21).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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